unstructured regions in proteins are known for mediating many protein-protein or protein-nucleotide interactions during regulation of transcription, translation, and cellular signal transduction. The question arises how conjugated glycans influence protein and peptide conformational dynamics and by that modify their biological activity. We compare the conformational dynamics of unstructured polypeptides consisting of eight glycine-serine repeat units with and without glycosylated serine units. We synthesized glycine-serine repeats with O-conjugated beta-galactose at every serine residue by solid-phase synthesis with glycosylated dipeptides as building blocks. Introducing an organic oxazine dye and tryptophan at either end of these peptides allows measurements of end-to-end contact kinetics. Upon van-der-Waals contact between dye and tryptophan fluorescence is quenched by photo induced electron transfer (PET). Fluorescence intensity fluctuations are analyzed using fluorescence correlation spectroscopy (FCS) and contact formation rate constants are determined. We studied influences from solvent viscosity and temperature on end-to-end contact formation rates and found a decrease of rate constants upon glycosylation. Arrhenius analysis of end-to-end contact rates yields enhanced activation energy for the glycosylated sample. The viscosity dependence of the relaxation rates shows that contact formation still is viscosity controlled. This study confirms previous reports that glycosylation has a significant influence on peptide dynamics mostly through steric hindrance.

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Transient Tertiary Contact Formation in the CGRP Neuropeptide Revealed by Nanosecond Laser Spectroscopy

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Calcitonin gene-related peptide (CGRP) is an intrinsically disordered, 37 residue neuropeptide that acts as a potent vasodilator, which is of considerable interest in migrane research. It is a member of the calcitonin peptide (Ct) family, together with amylin, calcitonin and adrenomedullin. These are genetically and structurally related intrinsically disordered hormone peptides that are able to bind to each other's receptors, though with varying degrees of affinity. They contain highly conserved sequence elements that have been experimentally shown to affect the secondary structural preferences of these peptides. The effect of such conserved elements on tertiary structure has not been experimentally explored to the same extent. Detecting tertiary structural properties of IDPs is considerably more challenging due to fast reconfigurations of the backbone over a wide range of possible conformations. High resolution time-resolved techniques are needed. We use a nanosecond-resolved spectroscopic technique based on tryptophan triplet quenching by cystine to detect tertiary contact formation in CGRP under varying solvent and temperature conditions. Using this technique, Vaiana et al.[1] have previously shown that conserved structural elements of amylin induce compact states characterized by short end-to-end distances, and that compaction is not driven by hydrophobic side-chains. Using triplet quenching we explore the effect of these conserved elements on the conformation and dynamics of CGRP, a peptide with higher mean hydrophobicity and net charge per residue than amylin. We discuss our findings in relation to secondary structural preferences of these peptides and discuss their possible functional role.

Footnote
[1] Vaiana S.M. et al. Biophys. J. 97 2009.

51-Plat

Single Molecule Study of the Intrinsically Disordered FG-repeat Nucleoporin 153

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The megaDalton sized nuclear pore complexes (NPCs) are among the largest molecular machines in eukaryotic cells. They span the nuclear envelope and constitute the only transport conduit between nucleoplasm and cytoplasm. Multiple copies of roughly a dozen different natively unfolded proteins form a selective permeability barrier inside the NPC and take a central role in the vital nucleocytoplasmic transport mechanism. Other than reoccurring phenylalanine-glycine elements (FG-repeats), these Nucleoporins (FG-Nups) have only limited sequence similarity among each other and across species. Still, they constitute a complex and distinct non-random amino acid (AA) architecture of FG-repeat clusters and intra-FG linkers. How such heterogeneous sequence composition relates to function and could actually give rise to a transport mechanism is still unclear. Currently a better understanding is largely hampered by our limited ability to study such highly flexible and intrinsically disordered proteins/protein domains. Here we describe a combined chemical biology and single molecule fluorescence approach to study the large human Nup153 FG-domain. in order to obtain insights into the properties of this domain beyond the average behavior, we probed the end-to-end distance (R_E) of several, approximately 50 residues long FG-repeat clusters in the context of the whole protein domain. Despite the sequence heterogeneity of these FG-clusters, we detected a reoccurring and consistent compaction from a relaxed coil behavior under denaturing conditions ($R_E/R_{E,RC} = 0.99 \pm 0.15$ with $R_{E,RC}$ corresponding to ideal relaxed coil behavior) to a collapsed state under native conditions ($R_E/R_{E,RC} = 0.79 \pm 0.09$). We then analyzed the properties of this protein on the supramolecular level, and determined that this human FG-domain was in fact able to form a hydrogel with physiological permeability barrier properties, i.e. nuclear transport cargos readily partition into the gel, while inert cargos do not.

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Electrostatics and Intrinsic Disorder: A Single-Molecule Study of the Sic1 Protein

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Intrinsically disordered proteins (IDPs) play critical yet often poorly understood roles in a variety of cellular processes. Despite an apparent antagonism between structural disorder and protein recognition, the large number of IDPs involved in protein regulation suggests that they could in fact provide advantages in recognition over well-folded proteins. Sic1 is an IDP inhibitor of a cyclin dependent kinase (CDK) in yeast, which interacts with a single site on its acceptor Cdc4 only upon phosphorylation of its multiple dispersed CDK sites. The multiple phosphorylation events can in principle be the basis for ultrasensitivity in protein-protein binding, however its molecular basis remains elusive[1].

We performed a systematic study of the Sic1's fluctuating conformations in different salt and denaturant concentrations using single-molecule Förster energy transfer (smFRET) and fluorescence correlation spectroscopy (FCS). smFRET data suggests that Sic1 protein is comprised of a continuum of conformers with varying end-end distances. Denaturant titration measurements suggest that these conformers are characterized by non-cooperative interactions. From FCS experiments, the exchange between Sic1 conformational states was found to occur on both ultrafast (10-100 ns timescale) and slow (10-100 ms) timescale. Burst smFRET experiments show that Sic1's end-end distances do not vary significantly upon addition of salt, which suggests that charge-shielding by salt may only affect the structure locally, around charged amino acids. Our single-molecule data resolves conformational heterogeneity and dynamics in a model IDP protein and represent the first step towards the validation of the polyelectrostatic model of the Sic1-Cdc4 interaction[2].

(1) Nash, P., Tang, X., Orlicky, S., Chen, Q., Gertler, F. B., Mendenhall, M. D., Sicheri, F., Pawson, T., Tyers, M. Nature 2001, 414, 514.
(2) Borg, M., Mittag, T., Pawson, T., Tyers, M., Forman-Kay, J. D., Chan, H. S. Proc. Natl. Acad. Sci. USA 2007, 104, 9650.

53-Plat

Integrating Theory, Simulations and Experiments to Reveal the Recognition-Specific Pathways in the Nuclear Co-Activator Binding Domain Ensemble

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The nuclear receptor co-activator binding domain (NCBD) selectively recruits transcription co-activators (TCAs) during the formation of the transcription pre-initiation complex. However, the biophysical mechanisms of NCBD:TCA recognition remain unclear as both NCBD and several of its corresponding TCAs are intrinsically disordered. We therefore probed the conformational diversity of the apo- and holo-forms of NCBD using all-atom, explicit solvent molecular dynamics simulations (~100 μ1/4s) and small-angle neutron scattering experiments. We integrated theory, simulations and experiments into a unified framework called anharmonic conformational analysis (ACA). ACA identifies a hierarchy of conformational sub-states, intermediates and pathways that play a key role in NCBD:TCA recognition. The transitions between sub-states can be modeled by a bent paperclip, whose arms correspond to helices, α1-α2-α3. The pathways reveal that α1 and α2 adopt conformations